Giving credit to residual bioresources: from municipal solid waste hydrolysate and waste plum juice to poly (3-hydroxybutyrate)

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Abstract

Municipal solid waste (MSW) is massively generated all over the world. Its organic fraction (OFMSW), which represents a high percentage of MSW, mainly contains biodegradable materials, namely food waste, paper and garden waste. The social cost of OFMSW treatment and/or disposal is a serious and widespread problem, particularly in highly populated areas. Thus, effective and innovative solutions, which include the upgrading of OFMSW, are being currently sought. In fact, the OFMSW abundance, availability and average composition suggest its considerable potential within the circular economy desideratum, paving the way to valorisation approaches. In this context, an OFMSW sugar-rich hydrolysate and its validation as a substrate for the production of the polyester poly(3-hydroxybutyrate) (P(3HB)), to date the only bioplastic easily biodegradable in marine environment, were successfully obtained in a previous study. Based on those results, this work addresses the upscaling of the fermentative production, in fed-batch mode, of P(3HB) by *Burkholderia sacchari*. The OFMSW hydrolysate was used as cultivation medium due to its balanced nutrient composition, while a plum waste juice, also rich in sugars, was applied as feed to the bioreactor. By implementing this strategy, a maximum P(3HB) production of $30 \text{ g} \cdot \text{L}^{-1}$ with an accumulation of $43\% \text{ g (P(3HB))} / \text{g cell dry weight (CDW)}$ after 51 h, was achieved. The use of the hydrolysate as initial medium resulted in higher CDW ($71 \text{ g} \cdot \text{L}^{-1}$) than that of the simulated hydrolysate ($62 \text{ g} \cdot \text{L}^{-1}$ in average), probably because the OFMSW hydrolysate favours biomass growth in detriment of P(3HB) production.

Keywords

42 Municipal solid waste; Bacterial cultivation; Fed-batch; *Burkholderia sacchari*; Poly(3-
43 hydroxybutyrate)

44 **Abbreviation list**

45 CDW, cell dry weight; CDW_{max} , maximum cell dry weight; DO, dissolved oxygen; EU,
46 European Union; GC-MS, gas chromatography-mass spectrometry; MSW, municipal
47 solid waste; OD_{600} , optical density at 600 nm; OFMSW, organic fraction of municipal
48 solid waste; P(3HB), poly(3-hydroxybutyrate); P(3HB) %, Accumulation percentage of
49 poly (3-hydroxybutyrate); $P(3HB)_{max}$, maximum production of poly (3-
50 hydroxybutyrate); PHAs, polyhydroxyalkanoates; PLA, polylactic acid; $Prod_{vol}$,
51 volumetric productivity; X_{res} , residual biomass; $Y_{p/s}$, yield of polymer on sugar
52 consumed.

1. Introduction

In the present days it is rare to find an object that does not contain a fraction of a petroleum-derived plastic, however small it may be. The excellent and versatile properties of plastics obtained by the petrochemical industry have led to an exponential increase in the scale of their production over the last decades. According to Lebreton et al. (2017), plastics production worldwide broke the barrier of 300 million tons in 2014 and it is foreseen that it will continue to increase in the forthcoming years. Moreover, their low price and ease of production favour the utilization of single-use plastics. This issue combined with their widespread resilience to biodegradation generates huge environmental concerns (Sardon and Dove, 2018). To date, disposal in land-fills and pyrolysis are the principal ways to manage plastic wastes. The later solutions only hinder the mitigation of the problem (Geyer et al., 2017). Recycling is another alternative, however only 5% of manufactured plastics are recycled. On the other hand, strategies such as reduction of their use and partial or total replacement by biodegradable polymers (bioplastics) are taking hold in recent years (Prata et al., 2019). The best known biopolymers are the aliphatic polyesters, namely polylactic acid (PLA) and the polyhydroxyalkanoates family (PHAs), polysaccharides (e.g. cellulose and starch) and some polypeptides (e.g. collagen) (Sabbah and Porta, 2017). In particular, PHAs, which are produced as intracellular carbon/energy storage under nutrient limitation (of e.g. nitrogen, phosphorous or oxygen) by a large variety of microorganisms, are attracting much attention because they can be tailored so as to offer similar properties to petroleum-derived polymers and, in addition, are completely biodegradable (Kabir et al., 2017). It is worth to

mention that PHAs are the only bioplastics that are degradable in the marine environment (Rujnić-Sokele and Pilipović, 2017; DiGregorio, 2009).

Among the PHA types, poly(3-hydroxybutyrate) (P(3HB)) is the most widely studied and is already used to make a large range of products such as packaging products, bottles and containers, electrical equipment, shopping bags, etc. (Misra et al., 2006; Sudesh et al., 2000; Albuquerque and Malafaia, 2018). However, the production cost of PHAs is considerably higher as compared to conventional plastics. At present, and as a result, society depends to a large extent on non-biodegradable polymers (Dietrich et al., 2017).

The major expenses in P(3HB) production by bacterial species are associated with the cost of the fermentation substrate, the maintenance of sterile conditions and the polymer extraction. In fact, the cost of the carbon source may account for 30% to 50% of the total production cost (de Paula et al., 2017; García et al., 2019; Obruca et al., 2015). Considering these facts, the key to obtain an economic carbon source appears to be the use of sugar rich substrates like e.g. hydrolysates of agricultural and industrial wastes or the organic fraction of municipal solid waste (OFMSW) (Yusuf, 2017; Ghanavati et al., 2015). In reality the OFMSW is one of the most abundant feedstocks in cities, accounting for approximately 30-40% of municipal solid waste (MSW) in Europe (Eurostat, 2018; Cesaro et al., 2019). Traditionally, most of this waste was deposited in landfills where its decomposition has been related to environmental problems such as gas emissions, leachate of pollutants and animal diseases, among others. Later, alternative forms of management were developed, which were more environmentally friendly, the most important being composting, mechanical-biological

treatment, biogas production and incineration (Kumar and Samadder, 2017; Trulli et al., 2018). Nevertheless, these strategies do often generate products with low added value to be considered economically feasible (Clarke, 2018). Nowadays, instead of a nuisance this type of waste is considered a valuable resource with which added-value products can be manufactured. Recently, Izaguirre et al. (2019) reported the production of P(3HB) by *Burkholderia sacchari* DSM 17165 using OFMSW as substrate and other authors used the same substrate to produce lipids, lactic acid, biofuels, volatile fatty acids, etc. (Gameiro et al., 2016; Ghanavati et al., 2015; López-Gómez et al., 2019; Mahmoodi et al., 2018).

The utilization of wastes to obtain new products reduces the environmental contamination, the greenhouse-gas emissions and the use of energy, and, moreover, meets the demands of a circular economy. In the last years this concept is gaining much attention and thus the European Union (EU) has adopted several initiatives outlined in the Circular Economy Action Plan (EC, 2015). This plan claims an increase in the MSW recycling rate and a reduction in landfilling. Bio-plastics production would not only alleviate the plastics pressure over the environment, but also reduce the amount of waste in landfills. To date and unfortunately these points have not been taken into consideration when the cost of bio-plastics is estimated, although they should be carefully considered when the life cycle analysis of plastics is addressed. According to (Vethaak and Leslie, 2016; Nizami et al., 2017; Guillard et al., 2018), the positive impact of the replacement of petroleum derived plastics by bioplastics would involve huge savings in environmental and health aspects.

This work is based on the preliminary data obtained by Izaguirre et al. (2019), where the production of P(3HB) was assessed at shaking flask scale using OFMSW hydrolysates as carbon source. The aim of the present study was the scaling-up of the process to a 5 L stirred tank reactor operated in the fed-batch-mode. The bacterial cells were grown on OFMSW as the only carbon source and the possibility of using plum concentrates as feed was evaluated. The results obtained are expected to shed light on the valorisation of urban solid wastes and help solve problems related to their management.

2. Materials and methods

2.1. Raw materials

2.1.1. OFMSW

The OFMSW samples were collected in November 2017 from EPELE composting plant, located in Gipuzkoa, Spain. They were formed by pre-screened household and garden wastes, and their treatment and composition has been recently described (Izaguirre et al., 2019).

2.1.2. Plum concentrate

A plum waste concentrate with a sugar composition of 120 g·L⁻¹ glucose and 60 g·L⁻¹ fructose was kindly provided by a bioresidues upgrader company (CATAR-CRIT Agroressources, France). This juice was obtained by thermo-mechanical extraction of plum wastes and was used as feed in some of the bioreactor fed-batch assays.

2.1.3. OFMSW hydrolysate preparation

To release the sugars from this feedstock a thermo-chemical pre-treatment followed by an enzymatic hydrolysis was carried out, as previously described by Izaguirre et al. (2019). Briefly, a 13.5% w/v OFMSW suspension was first pre-treated with H_2SO_4 $0.18 \text{ mol}\cdot\text{L}^{-1}$ at 121°C during 60 min using a 2 L stirred reactor (Duran GLS 80). The obtained suspension was chilled and the pH was raised to 4.8 with a solution of KOH $2 \text{ mol}\cdot\text{L}^{-1}$. As a second step, the dilute acid pre-treated OFMSW was enzymatically hydrolysed, using an enzyme mixture of Pentopan 500 BG, Celluclast BG and Glucoamylase NS 22035. The enzyme load per gram of substrate was 90 mg of Pentopan 500 BG, 150 mg of Celluclast BG and 108 mg of Glucoamylase NS 22035. The reaction was completed after 48 h, and the conditions of temperature, pH and agitation were 50°C , 4.8 and 170 rpm respectively. When the reaction ended, the residual pellet was removed from the sugar-rich solution by centrifugation at 10,000 rpm (Beckman Coulter Avanti J-26 XP), and the supernatant filtered using a $11 \mu\text{m}$ paper filter (Whatman). The resulting hydrolysate was sterilized in an autoclave (121°C , 20 min). Finally, the sugars composition of the hydrolysate was $21 \text{ g}\cdot\text{L}^{-1}$ glucose, $4 \text{ g}\cdot\text{L}^{-1}$ xylose and $0.6 \text{ g}\cdot\text{L}^{-1}$ arabinose.

2.2. Chemicals, microorganism, culture media and inoculum preparation

As described previously by Izaguirre et al. (2019), to preserve the strain *Burkholderia sacchari* DSM 17165, recently reclassified as *Paraburkholderia sacchari*, under optimal conditions, a culture bank was prepared in cryovials. These were kept at -80°C until their use as starter inocula.

The medium for the shake flask assays was formed by a simple salt medium as previously described (Izaguirre et al., 2019). Basal medium or OFMSW hydrolysate

were used to initiate the fed-batch fermentations. The basal medium consisting of a mixture of commercial sugars with the same composition as the hydrolysate was used as reference medium to validate the results using the hydrolysate for the production of P(3HB). As referred by Cesário et al. (2014), this medium was designed for phosphorus to be the first limiting nutrient. Salts and trace elements were obtained from Sigma-Aldrich (Steinheim, Germany; $\geq 99\%$ purity) and used as follows in the culture media (per litre): 4.0 g $(\text{NH}_4)_2\text{SO}_4$, 3.0 g KH_2PO_4 , 1.7 g citric acid, 40 mg EDTA, 1.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 10 mL of trace elements solution. The hydrolysate was supplemented with (per litre): 10 mL of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (100 g/L), 1.5 g KH_2PO_4 and 0.6 mL of trace elements solution in order to avoid nutritional deficiencies. The pH was adjusted to 6.8 with KOH (4 M). Concentrated juice of plum waste or solutions containing a blend of sugars simulating its composition were used as feed during the fed-batch phase.

The inocula (10% v/v) were prepared by inoculating one cryovial into a flask containing 250 mL of the mineral medium supplemented with 20 g/L of glucose and cultured for 12 h at 30 °C and 170 rpm in a rotary shaker (Infors HT, Ecotron) (Izaguirre et al., 2019).

2.3. Fed-batch production of P(3HB) using OFMSW hydrolysate

Fed-batch fermentations were carried out in a 5 L stirred BIOSTAT bioreactor (Sartorius AG, Germany) containing 2.5 L of basal medium or supplemented hydrolysate (or simulated hydrolysate when referred). Temperature was maintained constant at 32 °C using a recirculation cooler (Frigomix 1000; Sartorius AG, Germany). The dissolved oxygen (DO) set-point was 20% saturation. This value was maintained by cascade control of the stirring speed (initial speed was set at 250 rpm) and also by a constant air flow of 3.6 L·min⁻¹. The pH was controlled automatically at 6.8 with HCl (4 M) and

NH₄OH (20% v/v). The base served as nitrogen source throughout the fermentation, thus polymer accumulation was promoted by phosphate limitation. Foam generation during the fermentation was controlled through manual addition of antifoam (polypropylene glycol; Steinheim, Germany). Data were collected using the software MFCS DA (Sartorius AG, Germany). Samples (10 mL) were taken periodically for analysis of sugar and phosphorous consumption, cell growth and P(3HB) production. Pulse feeds of glucose (cultivation *A*), simulated plum concentrate (cultivations *B* and *C*) or real waste plum concentrate (cultivations *D* and *E*) were added manually when needed, based on the increase in the DO value.

2.4. Analytical methods

Bacterial growth was controlled by measuring the optical density (OD₆₀₀) of samples, taken throughout the fermentation, at 600 nm in a spectrophotometer (Shimadzu UV-2401PC). To determine the cell dry weight (CDW), 1.2 mL of culture broth was harvested by centrifugation (Sigma 1-15PK) at 14000 rpm for 5 min in 1.5 mL microtubes, previously dried and weighed. The liquid phase (1.0 mL) was stored at -20 °C for analyzing sugars and phosphate. The impurities of solid phase were eliminated by washing with distilled water (1 mL) and the resulting pellet was dried in an oven (Selecta 200) at 60 °C until constant weight. Dry cells were stored at 4 °C for further analysis of P(3HB) content.

Moisture and dry matter of the OFMSW were determined by heating the sample at 105 °C until constant weight.

209 The amount of sugars and phosphate in both, fermentation samples and OFMSW
210 hydrolysate, were analyzed using a liquid chromatography system (Agilent
211 Technologies 1260 Infinity II), equipped with an auto sampler (Agilent 1260
212 Vialsampler G7129A) and coupled to refraction index detector (Agilent 1260 RID
213 G7162A). The separation of the different compounds was performed on a Hi-Plex H
214 (300 mm x 7.7 mm) column. The mobile phase consisted of a 5 mM H₂SO₄ solution.
215 The operating conditions were: column temperature 65 °C, injection volume 2.4 µL and
216 flow rate 0.5 mL·min⁻¹. All analyses were performed in duplicate and standards were
217 used for identification and quantification of the compounds.

218 Total quantity of carbon, nitrogen and hydrogen was measured using a Leco CHNS-932
219 elemental analyzer.

220 To determine the P(3HB) concentration, 1.2 mL of samples from the culture broth
221 were taken and centrifuged. The cell pellet was subjected to methanolysis as
222 previously described by Izaguirre et al. (2019), and the monomers concentration
223 quantified by Gas Chromatography-Mass Spectrometry (GC-MS) using a 7890 A gas
224 chromatograph (Agilent Technologies, Avondale, PA, USA) coupled to a 5975 C
225 electron impact ionization mass spectrometer and 7683 B Agilent autosampler. The
226 chromatographic separation was carried out in the splitless mode (2 min at 120 °C) in a
227 HP-5MS UI capillary column (30 m × 0.25 mm, 0.25 µm, Agilent Technologies,
228 Avondale, PA, USA). The oven temperature program used for the separation was as
229 follows: the initial temperature was 40 °C, then increased at 10 °C·min⁻¹ until 60 °C,
230 after that maintained 4 min and finally increased at 50 °C·min⁻¹ until 230 °C. The helium
231 carrier gas flow was maintained constant at 1 mL·min⁻¹ and the injection volume was 2

μL. The qMS transfer line, ion source and quadrupole temperatures were 300 °C, 230 °C and 150 °C, respectively. Scan mode with mass range of 40-600 m/z was used for the measurements. The 3-methylhydroxybutyrate standard (Sigma Aldrich, Steinheim, Germany) was used for the calibration curves to quantify the polymer extracted.

3. Results and discussion

In recent years, the use of sugar-rich hydrolysates of waste materials in fermentation processes to produce high-value products such as P(3HB) has become popular. This fact originates from the need to reduce both the production cost of these products and the amount of wastes that are disposed in the environment. The hydrolysate used in this work was recently validated by Izaguirre et al. (2019) for P(3HB) production by *B. sacchari* DSM 17165. This strain has the ability to consume hexoses and pentoses simultaneously at specific rates that depend on the hexose/pentose ratio (Raposo et al., 2017). This is a valuable feature since the main sugars of the OFMSW hydrolysate are glucose (19 g·L⁻¹), xylose (3.4 g·L⁻¹) and arabinose (0.5 g·L⁻¹). The results obtained in the above cited work confirmed *B. sacchari* DSM 17165 as a good candidate for the bioreactor studies.

3.1. Fed-batch cultivations: preliminary study

Some recent studies where *B. sacchari* DSM 17165 was used for the production of P(3HB) have been published (Ashby et al., 2018; Cesário et al., 2014; de Sousa Dias et al., 2017; Rodríguez-Contreras et al., 2015). For example, Cesário et al. (2014), using a wheat straw hydrolysate as carbon source, achieved a biomass concentration of 136 g·L⁻¹ as well as a P(3HB) accumulation of 72% and a volumetric productivity of 1.5 g·(L·h)⁻¹ under phosphorous limitation. In most cases, this strain showed better

results under phosphorous rather than nitrogen limitation (Silva et al., 2004). In the light of the results obtained by these authors, polymer accumulation under phosphorous limitation was also the strategy followed in the present work.

To assess the behaviour of *B. sacchari* DSM 17165 during cultivations in the fed-batch mode, an assay was conducted (cultivation A) starting with the basal medium described in section 2.2. This medium was supplemented with 20 g·L⁻¹ of glucose as carbon source. During the cultivation, manual pulses of a concentrated glucose solution (500 g·L⁻¹) were added in order to maintain a high level of carbon and achieve high cell density. The time course of the CDW, P(3HB) concentration (g·L⁻¹), as well as the glucose and phosphorous concentrations in the medium are shown in Fig. 1.

A maximum CDW of 62 g·L⁻¹ was obtained after 47 h of fermentation. Phosphorus was depleted at 26 hours of cultivation time, after which polymer production increased rapidly to reach 38 g·L⁻¹. The yield of polymer on sugar consumed ($Y_{P/S}$) and the volumetric productivity obtained over the whole cultivation time ($Prod_{vol}$) were 0.20 (g_{P(3HB)}/g_{sugar}) and 0.81 g P(3HB)·L⁻¹·h⁻¹) respectively (Table 1). These values served as reference to assess the performance of subsequent cultivations using real hydrolysate or real plum concentrate.

3.2. Feeding strategies

Due to the limited reactor working volume, operation in the fed-batch mode requires the use of concentrated feed solutions to be able to reach high cell concentrations and also to prolong the production period. If the OFMSW hydrolysate was used as feed, it would be necessary to remove a portion of its water content in order to increase its sugar concentration. This additional step would imply high economic and energy costs.

However, some authors have highlighted the idea of using fruit waste juices as carbon source in ethanol, baker's yeast and bacterial cellulose production (Dhillon et al., 2013; Diboune et al., 2019; Kosseva, 2017). In this study, following the same approach, a waste plum concentrate was used as feed during the cultivation. As mentioned in section 2.1, the main sugars in the plum concentrate were glucose and fructose. This is a positive factor, since *B. sacchari* DSM 17165 is able to uptake fructose (Brämer et al., 2001).

Feed addition was performed differently during the day and during the night. During day time, the feed pulses were added manually when a decrease in sugar concentration or an increase in dissolved oxygen (DO) was detected in the culture medium. At night an external peristaltic pump was programmed to add pulses of 250 mL feed every 3.5 h, so that the culture medium always had an adequate concentration of carbon. The time interval between additions was set according to the sugar consumption rates which had been previously determined. This strategy turned out to be rather effective for P(3HB) production as discussed below.

3.3. Cell growth and polymer production in fed-batch cultivations

In order to obtain high productions of P(3HB), high cell concentrations should be reached during the initial phase of the culture. For that, the growing cells require a balanced medium composition where some nutrients, namely nitrogen, are essential. On this respect, the OFMSW hydrolysate has a rather complete nutrient composition, where in addition to previously mentioned sugars, other elements such as calcium, magnesium and nitrogen stand out (Izaguirre et al., 2019). Precisely, the high nitrogen content ($1.5 \text{ g}\cdot\text{L}^{-1}$) makes the hydrolysate particularly suitable for use as an initial

medium in the batch phase, since this element is essential for cell growth. On the other hand, to promote the production of P(3HB) the feed must have a high carbon content and at the same time have a limitation in at least one essential nutrient. As a matter of fact, the plum concentrate meets this criterion very well, since it has a high concentration of sugars and its content in nitrogen ($0.9 \text{ g}\cdot\text{L}^{-1}$) and phosphorus ($0.3 \text{ g}\cdot\text{L}^{-1}$) is low.

At bioreactor scale, prior to combining the use of the OFMSW hydrolysate as an initial medium and the plum concentrate as feed (cultivation *E*), experiments were conducted using mixtures of commercial sugars in which the composition of the hydrolysate (cultivations *B* and *D*) and of the plum concentrate (cultivations *B* and *C*) were simulated. The time course of CDW and P(3HB) produced as well as sugar and phosphate consumption for cultivations *B*, *C* and *D* is given on Fig 2. Rate and yield parameters calculated for each cultivation are given on Table 1.

Fig 2 shows that the rate of P(3HB) production when using simulated or real plum concentrate as feed (cultivations *B*, *C* and *D*) is lower as compared with cultivation *A* using glucose. This is because, even though glucose and fructose are consumed simultaneously, the rate of glucose consumption is higher than that of fructose, showing thus that *B. sacchari* has a clear preference for glucose as already reported by other authors (Nascimento et al., 2016). These results lead to lower polymer productivities in cultivations *B*, *C* and *D* as given in Table 1.

On the other hand, it is observed that when the hydrolysate is used as initial medium and simulated plum concentrate as feed (cultivation *C*), the attained CDW value is slightly higher ($65 \text{ g}\cdot\text{L}^{-1}$) than that obtained on the simulated hydrolysate (cultivations *B*

and *D*) (Fig. 2). This is clearly perceived already after 20 h cultivation. These observations, combined with the high values of residual biomass ($X_{res} = \text{CDW} - \text{Conc. P(3HB)}$) ($37.3 \text{ g}\cdot\text{L}^{-1}$) and of consumed sugars ($180 \text{ g}\cdot\text{L}^{-1}$) after 53 h, seem to indicate that the OFMSW hydrolysate promotes cell growth, disfavours polymer production. The lower yield of polymer on sugar, $Y_{P/S} = 0.15 \text{ g}_{\text{polymer}}\cdot\text{g}_{\text{sugar}}^{-1}$, calculated for cultivation C is an indication of that. Similar results were obtained by (Al-Battashi et al., 2018) using waste paper with *B. sacchari* DSM 17165. Also, it is interesting to see that with the real plum concentrate and simulated hydrolysate (cultivation *D*), the total amount of sugars consumed, the yield of polymer on sugar and the residual biomass are similar to those using the simulates (cultivation *B*). This fact indicates that the real plum concentrate does not negatively affect both the growth and production of P(3HB) by *B. sacchari*.

When the hydrolysate was used as initial medium and the real plum concentrate as feed solution (cultivation *E*), a maximum CDW of $71 \text{ g}\cdot\text{L}^{-1}$ after 51 h, with a P(3HB) accumulation of $30 \text{ g}\cdot\text{L}^{-1}$ was obtained (Fig. 3). In spite of the increase in CDW and P(3HB) production, the yield of polymer on sugars consumed ($Y_{P/S} (\text{g/g})$) was lower ($0.15 \text{ g}\cdot\text{g}^{-1}$, *c.f.* Table 1).

This can be ascribed to both the hydrolysate and plum concentrate favouring biomass production in detriment of P(3HB) accumulation, as indicated by the high value of residual biomass ($X_{res} = 41 \text{ g}\cdot\text{L}^{-1}$). The P(3HB) accumulation in cultivation *E* (43%) was comparable to the values achieved with the combination of i) real hydrolysate and simulated plum concentrate and ii) simulated hydrolysate and real plum concentrate. However, it was lower than with the combination of simulated hydrolysate and

simulated plum concentrate. It is worthy to note that the P(3HB) volumetric productivity in cultivation *E* ($0.59 \text{ g} \cdot (\text{L} \cdot \text{h})^{-1}$) attained very similar values to the ones obtained with the simulated initial medium and simulated feed (cultivation *B*).

This propensity to form biomass instead of polymer with the available sugars is most probably due to the N and P content, in particular in the OFMSW hydrolysate. To promote earlier polymer formation in the assays using OFMSW hydrolysate as substrate, the phosphorous content should be reduced in the supplemented mineral medium. Another reason for the preferential biomass formation when using real hydrolysates is the presence of peptides and amino acids derived from the hydrolysis of proteins in the OFMSW. For some microorganisms, these biomolecules are easier assimilated compared to an inorganic nitrogen source such as NH_4^+ . This could also explain why cell growth is faster with hydrolysates than with simulated media. A similar effect was reported by Al-Battashi et al. (2018), when assessing different sources of nitrogen in the production of P (3HB). They observed that the use of organic sources promoted the growth of the microorganism rather than the production of the polymer.

As pointed earlier, there are several reports on the use of *B. sacchari* strains to produce P(3HB) using hydrolysates as carbon source (Cesário et al., 2014; Silva et al., 2004; Pradella et al., 2010). The productivities and yields were generally higher than those reached in the present work. This could be due to the fact that they used lignocellulosic biomass of only a single type, while in this study a more complex and heterogeneous feedstock (OFMSW) was used. In contrast, the literature available on the use of complex substrates for P(3HB) production is limited. For example, Cerrone

et al. (2015) reported a P(3HB) production of $44.5 \text{ g}\cdot\text{L}^{-1}$ with a polymer content of 33% using mannitol rich ensiled grass press juice waste.

The results obtained in the present work suggest that the combination of the OFMSW hydrolysate as initial medium with the waste plum concentrate as feed solution is an attractive approach to produce P(3HB) from C-rich waste streams. However, it is still necessary to search for new ways to make the process more efficient towards P3HB production. The straight solution would be to adjust the phosphorous concentration supplemented to the hydrolysate so as to promote an earlier start of polymer production. Another possibility could be to extract the protein fraction of the residues prior to the saccharification of the carbohydrate fraction, applying thus the cascade biorefinery concept. This protein fraction could be upgraded separately to be included as additive in animal feed.

4. Conclusions

In this work P(3HB) was produced from an OFMSW hydrolysate and a residual plum juice stream using *B. sacchari* DSM 17165 at laboratory scale. Due to its balanced nutrient composition the OFMSW hydrolysate was used as initial cultivation medium to attain a high cell density and, subsequently, an off-stream of the production of plum concentrate, rich in sugars, was used as feed solution. The combination of these two feedstocks was shown to be an excellent strategy to reach a high P(3HB) productivity ($0.59 \text{ g}\cdot(\text{L}\cdot\text{h})^{-1}$). Further, it can be claimed that the combined utilization of the OFMSW hydrolysate and of the residual plum juice stream in P(3HB) production will not only decrease the amount of urban waste, but also reduce the environmental impact that would be originated if non-renewable raw materials were used. These results may

contribute to create sustainable solutions to manage the municipal waste and inspire the design of production plants based in bio-waste streams, hence contributing to a circular economy.

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References

- Al-Battashi, H, S., Verma, J.P., Al-Kindi, S., Al-Bahry, S., Annamalai, N., Sivakumar, N., Nair, A.S., 2018. Production of bioplastic (poly-3-hydroxybutyrate) using waste paper as a feedstock: Optimization of enzymatic hydrolysis and fermentation employing *Burkholderia sacchari*. *J. Clean. Prod.* 214, 236–247.
<https://doi.org/10.1016/j.jclepro.2018.12.239>
- Albuquerque, P.B.S., Malafaia, C.B., 2018. Perspectives on the production, structural characteristics and potential applications of bioplastics derived from polyhydroxyalkanoates. *Int. J. Biol. Macromol.* 107, 615–625.
<https://doi.org/10.1016/j.ijbiomac.2017.09.026>
- Ashby, R.D., Solaiman, D.K.Y., Nuñez, A., Strahan, G.D., Johnston, D.B., 2018. *Burkholderia sacchari* DSM 17165: A source of compositionally-tunable block-copolymeric short-chain poly(hydroxyalkanoates) from xylose and levulinic acid. *Bioresour. Technol.* 253, 333–342.
<https://doi.org/10.1016/j.biortech.2017.12.045>
- Brämer, C.O., Vandamme, P., Da Silva, L.F., Gomez, J.G.C., Steinbüchel, A., 2001. *Burkholderia sacchari* sp. nov., a polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil. *Int. J. Syst. Evol. Microbiol.* 51, 1709–1713. <https://doi.org/10.1099/00207713-51-5-1709>
- Cerrone, F., Davis, R., Kenny, S.T., Woods, T., O'Donovan, A., Gupta, V.K., Tuohy, M., Babu, R.P., O'Kiely, P., O'Connor, K., 2015. Use of a mannitol rich ensiled grass press juice (EGPJ) as a sole carbon source for polyhydroxyalkanoates (PHAs) production through high cell density cultivation. *Bioresour. Technol.* 191, 45–52.

427 <https://doi.org/10.1016/j.biortech.2015.04.128>

428 Cesário, M.T., Raposo, R.S., de Almeida, M.C.M.D., van Keulen, F., Ferreira, B.S., da
 429 Fonseca, M.M.R., 2014. Enhanced bioproduction of poly-3-hydroxybutyrate from
 430 wheat straw lignocellulosic hydrolysates. *N. Biotechnol.* 31, 104–113.
 431 <https://doi.org/10.1016/j.nbt.2013.10.004>

432 Cesaro, A., Conte, A., Belgiorno, V., Siciliano, A., Guida, M., 2019. The evolution of
 433 compost stability and maturity during the full-scale treatment of the organic
 434 fraction of municipal solid waste. *J. Environ. Manage.* 232, 264–270.
 435 <https://doi.org/10.1016/j.jenvman.2018.10.121>

436 Clarke, W.P., 2018. The uptake of anaerobic digestion for the organic fraction of
 437 municipal solid waste – Push versus pull factors. *Bioresour. Technol.* 249, 1040–
 438 1043. <https://doi.org/10.1016/j.biortech.2017.10.086>

439 de Paula, F.C., de Paula, C.B.C., Gomez, J.G.C., Steinbüchel, A., Contiero, J., 2017.
 440 Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) production from biodiesel by-
 441 product and propionic acid by mutant strains of *Pandoraea* sp. *Biotechnol. Prog.*
 442 33, 1077–1084. <https://doi.org/10.1002/btpr.2481>

443 de Sousa Dias, M.M., Koller, M., Puppi, D., Morelli, A., Chiellini, F., Braunegg, G., 2017.
 444 Fed-batch synthesis of poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-4-
 445 hydroxybutyrate) from sucrose and 4-hydroxybutyrate precursors by *Burkholderia*
 446 *sacchari* strain DSM 17165. *Bioengineering* 4.
 447 <https://doi.org/10.3390/bioengineering4020036>

448 Dhillon, G.S., Kaur, S., Brar, S.K., 2013. Perspective of apple processing wastes as low-

449 cost substrates for bioproduction of high value products: A review. *Renew.*
 450 *Sustain. Energy Rev.* 27, 789–805. <https://doi.org/10.1016/J.RSER.2013.06.046>
 451 Diboune, N., Nancib, A., Nancib, N., Aníbal, J., Boudrant, J., 2019. Utilization of prickly
 452 pear waste for baker's yeast production. *Biotechnol. Appl. Biochem.* 66, 744–754.
 453 <https://doi.org/10.1002/bab.1753>
 454 Dietrich, K., Dumont, M.J., Del Rio, L.F., Orsat, V., 2017. Producing PHAs in the
 455 bioeconomy — Towards a sustainable bioplastic. *Sustain. Prod. Consum.* 9, 58–70.
 456 <https://doi.org/10.1016/j.spc.2016.09.001>
 457 DiGregorio, B.E., 2009. Biobased Performance Bioplastic: Mirel. *Chem. Biol.* 16, 1–2.
 458 <https://doi.org/10.1016/j.chembiol.2009.01.001>
 459 EC – European Commission, 2015. Communication COM(2015) 614/2: closing the loop
 460 – An EU action plan for the Circular Economy [WWW Document]. URL [https://eur-](https://eur-lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-01aa75ed71a1.0012.02/DOC_1&format=PDF)
 461 [lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-](https://eur-lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-01aa75ed71a1.0012.02/DOC_1&format=PDF)
 462 [01aa75ed71a1.0012.02/DOC_1&format=PDF](https://eur-lex.europa.eu/resource.html?uri=cellar:8a8ef5e8-99a0-11e5-b3b7-01aa75ed71a1.0012.02/DOC_1&format=PDF)
 463 Eurostat, 2018. Municipal waste statistics [WWW Document]. URL
 464 [https://ec.europa.eu/eurostat/statistics-](https://ec.europa.eu/eurostat/statistics-explained/index.php/Municipal_waste_statistics)
 465 [explained/index.php/Municipal_waste_statistics](https://ec.europa.eu/eurostat/statistics-explained/index.php/Municipal_waste_statistics)
 466 Gameiro, T., Lopes, M., Marinho, R., Vergine, P., Nadais, H., Capela, I., 2016.
 467 Hydrolytic-Acidogenic Fermentation of Organic Solid Waste for Volatile Fatty
 468 Acids Production at Different Solids Concentrations and Alkalinity Addition.
 469 *Water. Air. Soil Pollut.* 227. <https://doi.org/10.1007/s11270-016-3086-6>
 470 García, A., Pérez, D., Castro, M., Urtuvia, V., Castillo, T., Díaz-Barrera, A., Espín, G.,

471 Peña, C., 2019. Production and recovery of poly-3-hydroxybutyrate [P(3HB)] of
 472 ultra-high molecular weight using fed-batch cultures of *Azotobacter vinelandii*
 473 OPNA strain. *J. Chem. Technol. Biotechnol.* 94, 1853–1860.
 474 <https://doi.org/10.1002/jctb.5959>

475 Geyer, R., Jambeck, J., Law, K., 2017. Production, Use, And Fate Of All Plastics Ever
 476 Made. *Sci. Adv.* 3, 25–29.

477 Ghanavati, H., Nahvi, I., Karimi, K., 2015. Organic fraction of municipal solid waste as a
 478 suitable feedstock for the production of lipid by oleaginous yeast *Cryptococcus*
 479 *aerius*. *Waste Manag.* 38, 141–148.
 480 <https://doi.org/10.1016/j.wasman.2014.12.007>

481 Guillard, V., Gaucel, S., Fornaciari, C., Angellier-Coussy, H., Buche, P., Gontard, N.,
 482 2018. The Next Generation of Sustainable Food Packaging to Preserve Our
 483 Environment in a Circular Economy Context. *Front. Nutr.* 5, 1–13.
 484 <https://doi.org/10.3389/fnut.2018.00121>

485 Izaguirre, J.K., da Fonseca, M.M.R., Fernandes, P., Villarán, M.C., Castañón, S., Cesário,
 486 M.T., 2019. Upgrading the organic fraction of municipal solid waste to poly(3-
 487 hydroxybutyrate). *Bioresour. Technol.* 290, 121785.
 488 <https://doi.org/10.1016/j.biortech.2019.121785>

489 Kabir, M.M., 2017. Can Bio-plastics Replace Non-Biodegradable Plastics? *J. Appl.*
 490 *Biotechnol. Bioeng.* 3, 381–383. <https://doi.org/10.15406/jabb.2017.03.00074>

491 Kosseva, M.R., 2017. Waste From Fruit Wine Production, *Science and Technology of*
 492 *Fruit Wine Production*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-800850->

493 8.00011-9

494 Kumar, A., Samadder, S.R., 2017. A review on technological options of waste to energy
495 for effective management of municipal solid waste. *Waste Manag.* 69, 407–422.
496 <https://doi.org/10.1016/j.wasman.2017.08.046>

497 Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J.,
498 2017. River plastic emissions to the world's oceans. *Nat. Commun.* 8, 1–10.
499 <https://doi.org/10.1038/ncomms15611>

500 López-Gómez, J.P., Latorre-Sánchez, M., Unger, P., Schneider, R., Lozano, C.C., Venus,
501 J., 2019. Assessing the organic fraction of municipal solid wastes for the
502 production of lactic acid. *Biochem. Eng. J.* 150, 107251.
503 <https://doi.org/10.1016/j.bej.2019.107251>

504 Mahmoodi, P., Karimi, K., Taherzadeh, M.J., 2018. Efficient conversion of municipal
505 solid waste to biofuel by simultaneous dilute-acid hydrolysis of starch and
506 pretreatment of lignocelluloses. *Energy Convers. Manag.* 166, 569–578.
507 <https://doi.org/10.1016/j.enconman.2018.04.067>

508 Misra, S.K., Valappil, S.P., Roy, I., Boccaccini, A.R., 2006. Polyhydroxyalkanoate
509 (PHA)/inorganic phase composites for tissue engineering applications.
510 *Biomacromolecules* 7, 2249–2258. <https://doi.org/10.1021/bm060317c>

511 Nascimento, V.M., Silva, L.F., Gomez, J.G.C., Fonseca, G.G., 2016. Growth of
512 *Burkholderia sacchari* LFM 101 cultivated in glucose, sucrose and glycerol at
513 different temperatures. *Sci. Agric.* 73, 429–433. [https://doi.org/10.1590/0103-](https://doi.org/10.1590/0103-9016-2015-0196)
514 [9016-2015-0196](https://doi.org/10.1590/0103-9016-2015-0196)

515 Nizami, A.S., Rehan, M., Waqas, M., Naqvi, M., Ouda, O.K.M., Shahzad, K., Miandad, R.,
 516 Khan, M.Z., Syamsiro, M., Ismail, I.M.I., Pant, D., 2017. Waste biorefineries:
 517 Enabling circular economies in developing countries. *Bioresour. Technol.* 241,
 518 1101–1117. <https://doi.org/10.1016/j.biortech.2017.05.097>

519 Obruca, S., Benesova, P., Marsalek, L., Marova, I., 2015. Use of lignocellulosic materials
 520 for PHA production. *Chem. Biochem. Eng. Q.* 29, 135–144.
 521 <https://doi.org/10.15255/CABEQ.2014.2253>

522 Pradella, J.G. da C., Taciro, M.K., Mateus, A.Y.P., 2010. High-cell-density poly (3-
 523 hydroxybutyrate) production from sucrose using *Burkholderia sacchari* culture in
 524 airlift bioreactor. *Bioresour. Technol.* 101, 8355–8360.
 525 <https://doi.org/10.1016/J.BIORTECH.2010.05.046>

526 Prata, J.C., Patrício Silva, A.L., da Costa, J.P., Mouneyrac, C., Walker, T.R., Duarte, A.C.,
 527 Rocha-Santos, T., 2019. Solutions and integrated strategies for the control and
 528 mitigation of plastic and microplastic pollution. *Int. J. Environ. Res. Public Health*
 529 16, 1–19. <https://doi.org/10.3390/ijerph16132411>

530 Raposo, R.S., de Almeida, M.C., de Oliveira, M. da C., da Fonseca, M.M., Cesário, M.T.,
 531 2017. A *Burkholderia sacchari* cell factory: production of poly-3-hydroxybutyrate,
 532 xylitol and xylonic acid from xylose-rich sugar mixtures. *N. Biotechnol.* 34, 12–22.
 533 <https://doi.org/10.1016/j.nbt.2016.10.001>

534 Rodríguez-Contreras, A., Koller, M., Miranda-de Sousa Dias, M., Calafell-Monfort, M.,
 535 Braunegg, G., Marqués-Calvo, M.S., 2015. Influence of glycerol on poly(3-
 536 hydroxybutyrate) production by *Cupriavidus necator* and *Burkholderia sacchari*.

537 Biochem. Eng. J. 94, 50–57. <https://doi.org/10.1016/j.bej.2014.11.007>
 538 Rujnić-Sokele, M., Pilipović, A., 2017. Challenges and opportunities of biodegradable
 539 plastics: A mini review. Waste Manag. Res. 35, 132–140.
 540 <https://doi.org/10.1177/0734242X16683272>
 541 Sabbah, M., Porta, R., 2017. Plastic Pollution and the Challenge of Bioplastics. J. Appl.
 542 Biotechnol. Bioeng. 2, 15406. <https://doi.org/10.15406/jabb.2017.02.00033>
 543 Sardon, H., Dove, A.P., 2018. Plastics recycling with a difference. Science (80-.). 360,
 544 380–381. <https://doi.org/10.1126/science.aat4997>
 545 Silva, L.F., Taciro, M.K., Michelin Ramos, M.E., Carter, J.M., Pradella, J.G.C., Gomez,
 546 J.G.C., 2004. Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose,
 547 glucose and sugarcane bagasse hydrolysate. J. Ind. Microbiol. Biotechnol. 31, 245–
 548 254. <https://doi.org/10.1007/s10295-004-0136-7>
 549 Sudesh, K., Abe, H., Doi, Y., 2000. Synthesis, structure and properties of
 550 polyhydroxyalkanoates: Biological polyesters. Prog. Polym. Sci. 25, 1503–1555.
 551 [https://doi.org/10.1016/S0079-6700\(00\)00035-6](https://doi.org/10.1016/S0079-6700(00)00035-6)
 552 Trulli, E., Ferronato, N., Torretta, V., Piscitelli, M., Masi, S., Mancini, I., 2018.
 553 Sustainable mechanical biological treatment of solid waste in urbanized areas
 554 with low recycling rates. Waste Manag. 71, 556–564.
 555 <https://doi.org/10.1016/j.wasman.2017.10.018>
 556 Vethaak, A.D., Leslie, H.A., 2016. Plastic Debris is a Human Health Issue. Environ. Sci.
 557 Technol. 50, 6825–6826. <https://doi.org/10.1021/acs.est.6b02569>

558 Yusuf, M., 2019. Agro-industrial waste materials and their recycled value-added
559 applications: Review. Handb. Ecomater. 4, 2699–2708.
560 https://doi.org/10.1007/978-3-319-68255-6_48

Fig. 1: Time course of CDW and P(3HB), glucose and phosphate concentrations during fed-batch cultivation A on mineral medium supplemented with 20 g·L⁻¹ of glucose; extra glucose was added manually in pulses.

Fig. 2: Time course of CDW, P(3HB), sugars and phosphate concentrations during fed-batch cultivations on a) basal medium supplemented with a simulated hydrolysate with pulses of simulated plum concentrate (cultivation B), b) OFMSW hydrolysate with pulses of simulated plum concentrate (cultivation C) and c) basal medium supplemented with a simulated hydrolysate with pulses of real plum concentrate (cultivation D).

Fig 3: Time course of CDW, P(3HB), phosphate and sugars concentrations during a fed-batch cultivation on real OFMSW hydrolysate with pulses of real waste plum concentrate (cultivation E).

576 **Table 1:** *Burkholderia sacchari* cultivation parameters calculated for each experiment based on the total sugar consumption, corresponding
577 biomass concentration and maximum polymer concentration.

Cultivation	Initial medium	Feed	Sugar consumed (g/L)	CDW _{max} (g/L)	P(3HB) _{max} (g/L)	X _{res} (g/L)	P(3HB) (%)	Time (h)	Prod _{vol} (g·(L·h) ⁻¹)	Y _{p/s} (g/g)
A	Basal	Glucose	155	62	38.0	23.8	61.0	47	0.81	0.20
B	Simulated hydrolysate	Simulated plum concentrate	160	63	31.5	31.5	50.0	52	0.61	0.20
C	Hydrolysate	Simulated plum concentrate	180	65	27.7	37.3	42.5	53	0.52	0.15
D	Simulated hydrolysate	Plum concentrate	150	61	27.8	33.2	45.4	54	0.51	0.19
E	Hydrolysate	Plum concentrate	195	71	30.0	41.0	43.0	51	0.59	0.15